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Screening for cervical abnormalities associated with EBV, HPV and HSV-2 infections in South-West Nigeria: A tale between sex and non-sex workers

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ABSTRACT

Aim: This study investigated socio-economic and clinical correlates of cervical abnormalities and some viral infections such as Human Papilloma virus (HPV), Epstein-Barr virus (EBV) and Herpes Simplex virus type 2 (HSV-2) among commercial sex workers (CSWs) and non-sex workers (NSWs) of reproductive age. It also estimated serum levels of SCCA1, p16 and Ki67 as potential non-invasive biomarkers of cervical abnormalities.

Methods: This cross-sectional study included 203 consenting participants (NSWs = 98 and CSWs = 105) within the age-range of 20–49 years. Interview based risk factor questionnaire was administered, Pap smears were made from cervical scrapings and classified accordingly while serum levels of SCCA1, Ki67 and p16, EBV, HPV and HSV2 antibodies were determined by ELISA method. Multivariate and multiple logistic regression analysis were on generated data carried out and significance set at $p < 0.05$.

Result: Among NSWs, higher prevalence of cervicitis, atypical squamous cell of undetermined significance (ASCUS), low grade squamous cell intraepithelial lesion (LSIL) and high grade squamous cell intraepithelial lesion (HSIL) were 18.4%, 16.3%, 11.2% and 8.2%, respectively while the prevalence of the cervical abnormalities were 7.6%, 7.6%, 8.6% and 3.8%, respectively among CSWs ($p = 0.04$). Decreasing and increasing sero-prevalence of viral infections were observed from cervicitis, ASCUS, LSIL to HSIL among NSWs (77, 56, 54.5 and 50%) and CSWs (75, 87.5, 100 and 100%), respectively ($p = 0.08$). When only ASCUS, LSIL and HSIL were considered, statistics showed p16 sensitivity and specificity of 64.7% and 79.5%, respectively (Odd ratio {OR} 0.7118), SCCA1 sensitivity and specificity of 83.8% and 97%, respectively (OR 166.9) and ki67 sensitivity and specificity of 63.2% and 94.0%, respectively (OR 26.83). It revealed that serum levels of SCCA1, ki67 and p16 were sensitivity to the classes of Pap smear at $p = 0.00$; $p = 0.00$ and $p = 0.01$, respectively.

Conclusion: This study suggests that while HPV infection could independently could promote the development of cervical abnormalities among CSWs, EBV infection and other factors may be potential factors among NSWs. It also suggests that serum levels of SCCA1, ki67 and p16 could be used as effective Pap smear complementary diagnostic biomarkers.

Abbreviations: Sex workers, (SWs); Non-sex workers, (NSWs); Epstein-Barr virus, (EBV); Herpes Simplex virus type 2, (HSV-2); Human Papilloma virus, (HPV); Human immunodeficiency virus, (HIV); High-grade squamous cell intraepithelial lesion, (HSIL); Low-grade squamous intraepithelial lesion, (LSIL); Atypical squamous cells of unknown significance, (ASCUS); Negative for intraepithelial lesion or malignancy, (NILM); Odds ratio, (OR); Odd ratio too large, (ORTL); Confidence interval, (CI); Multivariate analysis, (MANOVA); Not calculated, (NC); Sex work debut, (SWD); No formal education, (NFE); Multiple sexual partners, (MSP); Secondary, (Sec); Divorced/Separated/Widow, (D/S/W); Abnormal, (Abn); Participants, (Ppt); History of urogenital infection, (HUTI); Uptake of cervical screening, (UCS).

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1. Introduction

Being the third most recorded female cancers around the globe, cervical cancer represents approximately 10% of all female cancers[1] and 63% of all gynaecological cancers confirmed through biopsy.[2] The low uptake of Pap smear test (39%) due to lack of awareness (60%) accounts for the high prevalence and late diagnosis of cervical cancer in Nigeria.[3] Some of which are believed to be initiated by HPV, HSV2 and EBV infection.[4] Interestingly, HSV-2+EBV co-infection has been associated with vaginal lesions and anal intra-epithelial neoplasia.[5,6] But their roles in promoting malignant transformation of the cervical epithelium have not been extensively investigated. Studies suggest that the prevalence of HPV (20.1%), especially high-risk types, is higher in commercial sex workers (CSWs) compared with non-sex workers (NSWs).[7,8] The later suggests that SWs are more at risk of developing cervical lesions, though geographical variations may abound. The low sensitivity of Pap smears for high-grade squamous intraepithelial lesions (66%), large number of false positive test and variation of results between cytopathologists have intensified the search for a biomarker of high sensitivity and specificity.[9] Expressions of p16 and ki67 have been used in differentiating between dysplastic, and non-dysplastic tumours.[10,11] while serum squamous cell carcinoma antigen (SCCA) has been used to differentiate between lymph node metastasizing and non-metastasizing vulva and cervical cancer.[12,13] The study determined the prevalence of abnormal Pap smears, EBV, HPV and HSV2 among CSWs and NSWs. It also explored the use of serum ki67, p16 and SCCA1 expression as complementary Pap smears diagnostic biomarkers.

2. Material and methods

2.1. Study setting and design

This cross-sectional study was carried out between the month of December 2016 and August 2017 in Sagamu and Abeokuta, Ogun State. A total of 250 questionnaires (125 for CSWs and NSWs) were administered to obtain the socio-demographic and clinical characteristics from consenting participants. Only direct (full-time) sex workers who were registered with the management of some selected brothels and club houses were included in this study.[14] More so, age matched non-sex workers who were sexually active were also included. Following administration of interview based questionnaire, participants ($n = 32$) who were identified to be HIV positive, pregnant, menstruating, menopausal, postmenopausal or had had hysterectomy were excluded from this study. Out of the 216 participants screened for cervical lesions, 13 had inadequate Pap smears and thus were excluded from the study. The 203 remaining participants (98 non-sex workers [mean age $= 36.42 \pm 9.30$ years] and 105 sex workers [mean age $= 31.25 \pm 7.46$ years]), within the age range of 20–49 years, were grouped age-wise: 20–29, 30–39 and 40–49years. Factors such as residency (urban vs rural), marital status (single vs married), tribes (Igbo, Yoruba, Hausa and others), education level, income status (low= < N18,000 [minimum wage] and high= > N54,000) use of contraceptives, history of urogenital infection, itching around the vulva, parity, vaginal bleeding after sex, previous cervical screening, sexual abuse, alcohol consumption, and smoking status, among others were recorded for each patient.

2.2. Blood sample collection and assays

Sera were separated from whole-blood samples after 2 h of collection and preserved at −20 °C until when analyzed. Samples were investigated for viral IgG and IgM antibodies using kits (from Calbiotech Inc, El Cajon, USA; Qingdao Hightop Biotech Co. Ltd, China). Samples were also investigated for Human Squamous Cell Carcinoma Antigen 1 (SCCA1), Ki-67 antigen and p16 by ELISA method (using kits from Melsin Medical Co. Ltd, China and Bioassay Technology Laboratory, Shanghai, China; Elabscience Biotechnology Inc, USA, respectively according to manufacturers' instruction). Cut-off point for SCCA1, Ki67 and p16 positivity were 1650 pg/ml, 7849 ng/ml and 8100 ng/L, respectively.

2.3. Sample collection, handling and classification of smears

Pap smears were made following colposcopy assisted cervical scrapping using Ayres spatula after dilating the vagina with lubricated disposable speculum. Smears were stained by Papanicolaou's technique and classified based on the Bethesda system: 1. High-grade squamous cell intraepithelial lesion (HSIL), 2. Low-grade squamous intraepithelial lesion (LSIL), 3. Atypical squamous cells of unknown significance (ASCUS) and 4. Negative for intraepithelial lesion or malignancy (NILM). Participants whose Pap smear result were consistent with ASCUS, LSIL and HSIL (abnormal Pap smears) were counseled and referred to gynecologist who initiated a follow-up plan for those whose Pap smears were consistent with ASCUS to monitor their progression or regression. Those whose Pap smears were consistent with LSIL and HSIL were biopsied and treated using colposcopy assisted loop electrosurgical excision procedure (LEEP) following the application of vinegary liquid (to exposure diseased areas). A 6 month follow-up plan was also initiated to monitor the re-occurrence of the abnormalities. Tissues sections were stained by H&E technique and immunohistochemical technique to demonstrate HPV and Ki67 positivity using Avidin–Biotin Immunoperoxidase method (Abcam, Nigeria). Mounted stained sections were scored by two experienced Histopathologists.

2.4. Ethical consideration

Ethical clearance was sort from State Hospital Abeokuta Research Ethics Services (SHA/RES/VOL.2/177).

2.5. Statistical analysis

Data generated were subjected to Analysis of variance (ANOVA), Chi-square (Fisher's exact) test for trends, and Paired T-test using GraphPad Prism (Version 7.03). Significant levels were set at $p < 0.01$ and $p < 0.05$. Multivariate analysis (MANOVA) and multiple logistic regression analysis were also carried out to assess the associations between some demographics, viral infections and cervical abnormalities.

3. Results

This is the first study to elucidate the frequency of abnormal Pap smears in relation to EBV, HPV and HSV-2 infections among CSWs and NSWs in Ogun State, Western Nigeria. It delineates the several factors that may influence the acquisition of onco-viruses and development of cervical malignancy. It also evaluates the effectiveness of using serum expression of ki67, p16, and SCCA1 to distinctly differentiate between one class of Pap smear from another in order to reduce inter- and intra-observer variability among cytopathologists.

3.1. Prevalence of cervical abnormalities

Among NSWs, higher prevalence of cervicitis, atypical

squamous cell of undetermined significance (ASCUS), low grade squamous cell intraepithelial lesion (LSIL) and high grade squamous cell intraepithelial lesion (HSIL) were 18.4%, 16.3%, 11.2% and 8.2%, respectively while the prevalence of such cervical abnormalities among CSWs were 7.6%, 7.6%, 8.6% and 3.8%, respectively at $p = 0.04$ (Fig. 1). Decreasing and increasing prevalence of viral infections were observed from cervicitis, ASCUS, LSIL and HSIL among NSWs (77, 56, 54.5 and 50%) and sex workers (75, 87.5, 100 and 100%), respectively ($p = 0.08$).

3.2. Frequency of viral infection

The prevalence of EBV infection was significantly higher among NSWs (40.8%) when compared with CSWs (23.8%) at $p = 0.02$, while the prevalence of HPV infection was higher in CSWs (37.1%) when compared with NSWs (24.5%) at $p = 0.09$. The prevalence of HSV-2 infection was slightly lower in NSWs (49%) compared with CSWs (49.5%) at $p = 0.77$. Among CSWs, the prevalence of EBV, HPV and HSV-2 mono-infections, EBV + HSV-2 and HPV + HSV-2 bi-infection and EBV + HPV + HSV-2 tri-infection were 2.9, 14.3, 20, 6.7, 10.5 and 12.3%, respectively while that of NSWs were 3.1, 4.1, 11.2, 22.4, 5.1 and 15.3%, respectively ($p = 0.82$). Among CSWs with normal Pap smear, the prevalence of EBV, HPV and HSV-2 mono-infections, EBV + HSV-2 and HPV + HSV-2 bi-infection and EBV + HPV + HSV-2 tri-infection were 3.9, 18.4, 19.7, 5.3, 7.9 and 5.3%, respectively while CSWs with abnormal Pap smear had 0.0, 3.4, 20.7, 17.2, 17.2 and 31.0%, respectively ($p = 0.44$). Among NSWs with normal Pap smear, the prevalence of EBV, HPV and HSV-2 mono-infections, EBV + HSV-2 and HPV + HSV-2 bi-infection and EBV + HPV + HSV-2 tri-infection were 0.0, 4.4, 11.1, 26.7, 2.2 and 15.6%, respectively while NSWs abnormal Pap smear had 5.7, 3.8, 11.3, 18.9, 7.5 and 15.1%, respectively ($p = 0.62$). Overall, results showed that viral mono-infections were relatively higher among those whose Pap smears were consistent with ASCUS. Among both groups, significant positive correlation was observed between serum levels of HSV-2 IgG and EBV IgG ($p = 0.00$), HSV-2 IgM and EBV IgG ($p = 0.00$) and HSV-2IgM and age ($p = 0.03$).

3.3. Socio-clinical characteristics among non-sex workers and sex workers

Among NSWs, MANOVA showed significant relationship between marital status, education, income status, parity, contraceptives, itching around the genitalia, vaginal discharge, uptake of cervical screening and cervical integrity (Pap smear) at $p < 0.05$. Table 1 shows that among NSWs, participants within the age range of 30–39 years, Muslims, middle income earners and those who had had previous cervical screening were significantly less likely to have abnormal Pap smear, while those who use condom (85% of the unmarried and D/S/W, 51.1% of those who had multiple sexual partners), those who itch around their vulva and experience vaginal discharge (suggestive of current ongoing infection) were significantly more likely to have abnormal Pap smear ($p < 0.05$; multiple logistic regression). None of the NSWs used condom consistently during sex intercourse. Among CSWs, MANOVA showed significant relationship between age range, tribe, religion, parity, vaginal discharge, number of clients/month, viral infections and cervical integrity (Pap smear) at $p < 0.05$. MANOVA also showed significant relationship between income status, sexual abuse, cervical integrity (Pap smear) and viral infections at $p < 0.05$. Table 2a shows decreasing viral infection and cervical abnormalities with increase in age of sex workers. Table 2b shows increasing frequency of viral infection from cervicitis to HSIL. In other words, it shows increasing cervical abnormalities with increasing viral co-infection, especially in HPV + HSV-2 and EBV + HPV + HSV-2 co-infections. Table 2 shows that shows that middle income earners, those who had been delivered of 1–2 children, those with viral mono-infections and EBV + HSV-2 co-infection were significantly less likely to have abnormal Pap smear compared with their respective reference groups ($p < 0.05$; multiple logistic regression). It reveals increasing frequency of abnormal Pap smears with higher number of sex working clients.

In relation socio-economic and clinical demographics, the figure shows significant differences in the occurrence of viral mono-infections and co-infections (except for EBV + HPV + HSV-2 co-infection) between sex workers and non-sex workers ($p < 0.05$; *t*-test). It also reveals lower frequency of EBV mono-infections

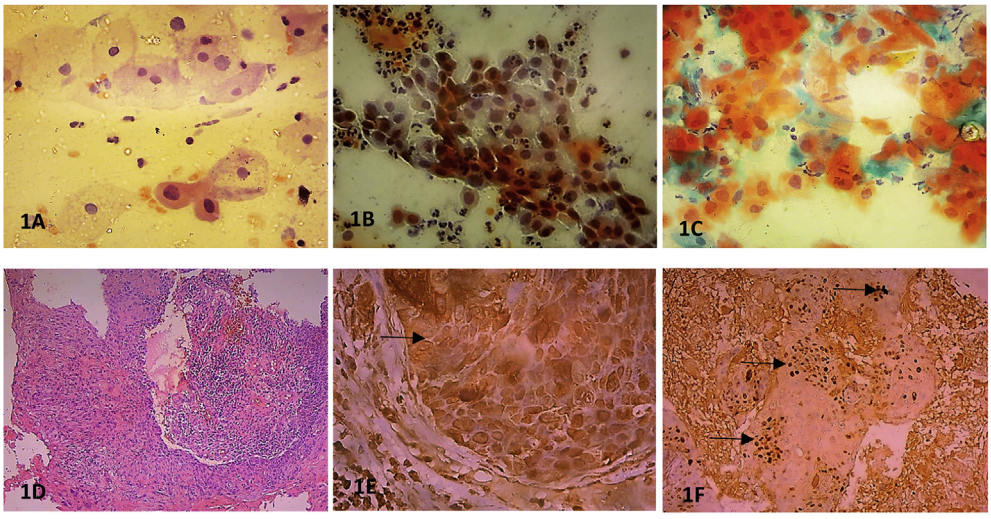

Fig. 1. AIndicates ASCUS with evidence of atypical cells, few polymorphs and a few red cells (X400). Fig. 1B: LSIL with abundant polymorphs and red cells, a few cells with moderately increased hyperchromatic nuclei (X400). Fig. 1C: HSIL with evidence of increased nuclear cytoplasmic ratio, hyperchromatic nuclei and a few polymorphs (X400). Fig. 1D: Section with evidence of cervical intraepithelial lesion III (CINIII; X100). Fig. 1E: CIN III showing intense cytoplasmic staining depicting HPV positivity (X400). Fig. 1F: CIN III showing intense positive nuclear staining (deep brown) for ki67 (X100).

Table 1
Behavioural and demographics characteristics of NSWs in relation to viral infection and abnormal Pap smear.

Variables	Sub-Variable	No of Ppt. n = 98	No of Abn smears n = 53	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 60	OR' (95% CI) Viral infection	P-value	MANOVA
Age range	20–29	21	17 (81.0)	1	0.03*		10 (47.6)	1	0.36	
	30–39	54	24 (44.4)	0.22 (0.06–0.84)	0.05*	F = 1.85	35 (64.8)	0.49 (0.49–1.63)	0.24	F = 0.59
	40–49	23	12 (52.2)	1.15 (0.43–3.05)	0.53	P = 0.13	15 (65.2)	0.98 (0.35–2.74)	0.97	P = 0.74
Marital Status	Unmarried	19	15 (78.9)	1	0.06		10 (52.6)	1	0.72	
	Married	78	37 (47.4)	ORTL	1.00	F = 3.08	49 (62.8)	ORTL	1.00	F = 0.87
	D/S/W	1	1 (100)	ORTL	1.00	P = 0.02*	1 (100)	ORTL	1.00	P = 0.52
Tribe	Igbo	11	5 (45.5)	1	0.84		6 (54.5)	1	0.90	
	Yoruba	86	47 (54.7)	ORTL	1.00	F = 1.25	53 (61.6)	ORTL	1.00	F = 1.05
	Hausa	1	1 (100)	ORTL	1.00	P = 0.29	1 (100)	ORTL	1.00	P = 0.40
Education	N.F.E.	3	3 (100)	1	0.82		3 (100)	1	0.98	
	Primary	9	6 (66.7)	ORTL	1.00		6 (66.7)	ORTL	1.00	
	Secondary	27	13 (48.1)	1.81 (0.41–7.91)	0.43	F = 0.00	16 (59.3)	1.37 (0.31–6.03)	0.68	F = 1.29
	Post Sec	59	31 (52.5)	0.84 (0.45–2.77)	0.84	P = 0.10	35 (59.3)	1.00 (0.40–2.52)	1.00	P = 0.27
Residence	Urban	80	43 (53.8)	1		F = 1.93	47 (58.8)	1		F = 1.67
	Rural	18	10 (55.6)	0.93 (0.33–2.60)	0.89	P = 0.11	13 (72.2)	0.55 (0.18–1.69)	0.29	P = 0.14
Religion	Christian	76	37 (48.7)	1		F = 2.05	48 (63.2)	1		F = 0.35
	Muslim	22	16 (72.7)	0.36 (0.13–1.01)	0.05*	P = 0.09	12 (54.5)	1.43 (0.54–3.73)	0.47	P = 0.91
Income status	Low	31	23 (74.2)	1	0.02*		16 (51.6)		0.38	
	Middle	46	21 (45.7)	3.83 (1.18–12.48)	0.01*	F = 5.90	31 (67.4)	0.66 (0.21–2.03)	0.46	F = 0.25
	High	21	9 (42.9)	1.12 (0.40–3.17)	0.12	P = 0.00*	13 (61.9)	1.27 (0.43–3.73)	0.66	P = 0.96
Parity	Null	21	13 (61.9)	1	0.64		10 (47.6)	1	0.36	
	1-2 Children	9	4 (44.4)	1.44 (0.53–3.93)	0.42	F = 2.58	6 (66.7)	0.50 (0.18–1.34)	0.17	F = 0.79
	Multiparous	68	36 (52.9)	0.71 (0.18–2.88)	0.54	P = 0.04*	41 (60.3)	1.10 (0.18–1.34)	0.91	P = 0.58
Age at first sex	Less 18	20	7 (35.0)	1	0.07		12 (60.0)	1	0.87	
	19–21	34	23 (67.6)	0.49 (0.17–1.47)	0.20	F = 2.58	22 (64.7)	1.04 (0.35–3.05)	0.95	F = 0.96
	22 and above	44	23 (52.7)	1.91 (0.75–4.84)	0.17	P = 0.27	26 (59.1)	1.27 (0.50–3.20)	0.61	P = 0.46
Contraceptives	Hormonal	70	31 (44.3)	1		F = 8.30	45 (64.3)	1		F = 0.56
	Condom	28	22 (78.6)	4.79 (1.72–13.31)	0.00*	P = 0.00*	15 (68.2)	1.56 (0.64–3.80)	0.40	P = 0.76
Sex Abuse	No	82	46 (56.1)	1		F = 1.62	47 (57.3)	1		F = 0.78
	Yes	16	7 (43.8)	1.88 (0.65–5.43)	0.25	P = 0.18	10 (62.5)	3.23 (0.85–12.20)	0.08	P = 0.59

Variables	Sub-Variable	No of Ppt. n = 98	No of Abn smears n = 53	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 60	OR' (95% CI) Viral infection	P-value	MANOVA
Oral Sex	No	83	44 (53.0)	1		F = 1.18	51 (61.4)	1		F = 0.36
	Yes	15	9 (60.0)	0.61 (0.19–1.98)	0.42	P = 0.33	9 (60.0)	0.22 (0.47–1.06)	0.06	P = 0.91
Abortion	No	19	10 (52.6)	1		F = 1.48	11 (57.9)	1		F = 0.52
	Yes	79	43 (54.4)	1.08 (0.39–2.93)	0.88	P = 0.22	49 (62.3)	0.35 (0.11–1.16)	0.09	P = 0.79
Smokers	No	95	52 (54.7)	1		F = 1.00	58 (61.1)			F = 0.24
	Yes	3	1 (33.3)	2.42 (0.20–27.59)	0.48	P = 0.41	2 (61.1)	0.78 (0.07–8.95)	0.85	P = 0.93
Alcohol	No	85	33 (38.8)	1		F = 1.32	51 (60.0)			F = 0.53
	Yes	13	8 (61.5)	0.70 (0.21–2.33)	0.56	P = 0.27	9 (69.2)	2.03 (0.63–6.59)	0.24	P = 0.78
M.S.P.	No	80	43 (53.8)	1		F = 2.31	48 (60.0)			F = 1.58
	Yes	18	10 (55.6)	1.08 (0.39–2.93)	0.89	P = 0.06	12 (66.7)	1.55 (0.57–4.26)	0.39	P = 0.16
H.UT.I.	No	75	43 (57.3)	1		F = 1.89	45 (60.0)	1		F = 0.80
	Yes	23	10 (43.4)	1.75 (0.65–4.24)	0.25	P = 0.12	15 (65.2)	0.80 (0.30–2.12)	0.65	P = 0.57
Ulcer/Warts	No	94	51 (54.3)	1		F = 1.09	58 (61.7)			F = 0.31
	Yes	4	2 (50.0)	1.19 (0.31–23.10)	0.87	P = 0.37	2 (50.0)	5.06 (0.50–50.52)	0.17	P = 0.93
Vulval itching	No	23	3 (13.0)			F = 6.35	13 (56.5)			F = 0.51
	Yes	75	50 (66.7)	13.33 (3.62–49.17)	0.00*	P = 0.00*	46 (61.3)	0.96 (0.37 2.51)	0.94	P = 0.80
Discharge	No	47	33 (48.9)	1		F = 3.6.4	28 (59.6)			F = 0.61
	Yes	51	20 (39.2)	3.65 (1.58–8.47)	0.00*	P = 0.01*	32 (62.7)	0.80 (0.36–1.80)	0.59	P = 0.73
Vaginal bleeding	No	88	47 (53.4)	1		F = 0.82	53 (60.2)			F = 0.82
	Yes	10	6 (60.0)	0.46 (0.10–2.23)	0.34	P = 0.51	8 (80.0)	0.62 (0.15–2.56)	0.51	P = 0.56
Pain during Sex	No	90	50 (55.6)	1		F = 1.41	54 (60.0)			F = 1.04
	Yes	8	3 (37.5)	1.40 (0.24–8.09)	0.71	P = 0.24	6 (75.0)	0.48 (0.09–2.50)	0.38	P = 0.40
Pelvic Pain	No	79	44 (55.7)	1		F = 2.17	47 (59.5)			F = 0.51
	Yes	19	9 (47.4)	1.39 (0.51–3.81)	0.51	P = 0.08	11 (57.9)	1.13 (0.41–3.11)	0.82	P = 0.80
U.C.S.	No	81	40 (49.4)	1		F = 1.86	49 (60.5)			F = 1.62
	Yes	17	13 (76.5)	0.30 (0.09–1.00)	0.05*	P = 0.12	11 (64.7)	0.79 (0.27–2.36)	0.68	P = 0.15

Variables	Sub-Variable	No of Ppt. n = 98	Nº No of Abn smears n = 53	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 60	OR' (95% CI) Viral infection	P-value	MANOVA
Viral Infection	No infection	38	20 (52.6)	1	0.94					
	EBV	3	3 (100)	ORTL	1.00					
	HPV	4	2 (50.0)	0.90 (0.12–7.07)	0.92					
	HSV-2	11	6 (54.5)	1.08 (0.28–4.15)	0.91					
	EBV + HSV-2	22	10 (45.5)	0.75 (0.26–2.15)	0.59					
	HPV + HSV-2	5	4 (80.0)	3.60 (0.37–35.27)	0.27	F = 0.79				
	EBV + HPV + HSV-2	15	8 (53.3)	1.03 (0.31–3.41)	0.96	P = 0.54				
Cervical Integrity	Normal	45					27 (60.0)	1	0.60	
	Cervicitis	18					14 (77.8)	1.50 (0.33–6.78)	0.60	
	ASCUS	16					9 (56.3)	3.50 (0.59–20.68)	0.17	
	LSIL	11					6 (54.5)	1.30 (0.23–7.05)	0.77	F = 0.35
	HSIL	8					4 (50.0)	1.2 (0.19–7.44)	0.85	P = 0.91

Keys: OR; Odds ratio, ORTL; Odd ratio too large, CI; Confidence interval, MANOVA; Multivariate analysis, NC; Not calculated (calculation included empty cells), SWD; Sex work debut, N.F.E; No formal education, M.S.P; Multiple sexual partners, Sec; Secondary; D/S/W; Divorced/Separated/Widow, Abn; Abnormal, Ppt; participants, HUTI; History of urogenital infection; Uptake of cervical screening.

Table 2
A: Behavioural and demographics characteristics of CSWs in relation to viral infection and abnormal Pap smear.

Variables	Sub-Variable	No of Ppt. n = 105	No of Abn smears n = 29	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 72	OR' (95% CI) Viral infection	P-value	MANOVA
Age range	20–29	63	19 (30.2)	1	0.46		45 (71.4)	1	0.69	
	30–39	32	9 (28.1)	3.89 (0.46–32.86)	0.21	F = 2.94	21 (65.6)	1.54 (0.39–6.12)	0.54	F = 1.42
	40–49	10	1 (10.0)	3.52 (0.39–31.94)	0.26	P = 0.02*	6 (60.0)	1.11 (0.26–4.75)	0.89	P = 0.22
Marital Status	Unmarried	68	16 (23.5)	1	0.34		47 (69.1)	1	0.38	
	Married	17	7 (41.1)	0.72 (0.24–2.18)	0.56	F = 0.82	14 (82.4)	1.71 (0.62–4.73)	0.30	F = 1.93
	D/S/W	20	6 (30.0)	1.63 (0.42–6.36)	0.48	P = 0.52	11 (55.0)	2.66 (0.64–11.06)	0.18	P = 0.08
Tribe	Igbo	37	9 (24.3)	1	0.88		30 (81.1)	1	0.11	
	Yoruba	18	6 (33.3)	0.78 (0.28–2.13)	0.62		11 (61.1)	2.74 (0.98–7.72)	0.06	
	Hausa	9	2 (22.2)	1.21 (0.37–3.97)	0.76	F = 4.69	5 (55.6)	1.01 (0.32–3.13)	0.99	F = 1.30
	Others	41	12 (29.0)	0.69 (0.13–3.82)	0.67	P = 0.00*	26 (63.4)	0.51 (0.12–2.20)	0.37	P = 0.27
Education	N.F.E.	10	2 (20.0)	1	0.06		7 (70.0)	1	0.97	
	Primary	34	16 (47.1)	ORTL	1.00		22 (64.7)	0.00	1.00	
	Secondary	55	11 (20.0)	0.00	1.00	F = 1.50	36 (65.5)	0.00	1.00	F = 0.82
	Post Sec	6	0 (0.0)	0.00	1.00	P = 0.21	6 (100)	ORTL	1.00	P = 0.56
Residence	Urban	46	13 (28.3)	1		F = 0.42	33 (71.7)	1		F = 0.87
	Rural	59	16 (27.1)	1.05 (0.45–2.50)	0.90	P = 0.79	35 (59.3)	1.51 (0.66–3.46)	0.33	P = 0.52
Religion	Christian	100	26 (26.0)	1		F = 2.45	69 (69.0)	1		F = 0.26
	Muslim	5	3 (60.0)	0.23 (0.04–1.48)	0.12	P = 0.05*	3 (60.0)	3.19 (0.22–3.19)	0.22	P = 0.96
Income status	Low	38	5 (13.2)	1	0.10		27 (71.1)	1	0.63	
	Middle	53	17 (32.1)	0.25 (0.06–0.99)	0.05*	F = 1.32	34 (64.2)	0.98 (0.25–3.81)	0.98	F = 2.98
	High	14	6 (42.9)	0.63 (0.19–2.10)	0.45	P = 0.27	11 (78.6)	0.66 (0.18–2.39)	0.53	P = 0.01*
Parity	Null	61	13 (21.3)	1	0.11		42 (68.9)	1	0.46	
	1-2 Children	5	0 (0.0)	0.39 (1.16–0.94)	0.04*	F = 2.64	2 (40.0)	0.91 (0.38–2.16)	0.83	F = 0.77
	Multiparous	39	16 (41.0)	ORTL	1.00	P = 0.04*	28 (71.8)	0.30 (0.04–2.01)	0.21	P = 0.59
Contraception	Condom	105	29 (27.6)	NC	NC		72 (68.6)	NC	NC	
Sexual Abuse	No	84	24 (28.6)	1		F = 0.36	57 (67.9)	1		F = 2.45
	Yes	21	5 (23.8)	0.83 (0.27–2.52)	0.74	P = 0.84	15 (71.4)	0.76 (0.27–2.16)	0.61	P = 0.03*
Oral Sex	No	84	22 (26.2)	1		F = 0.49	60 (71.4)	1		F = 0.68
	Yes	21	7 (33.3)	0.887 (0.31–2.57)	0.83	P = 0.74	12 (57.1)	1.88 (0.70–5.02)	0.21	P = 0.67
Abortion	No	35	11 (31.4)	1		F = 2.37	20 (57.1)	1		F = 1.95
	Yes	70	18 (25.7)	1.43 (0.58–3.51)	0.44	P = 0.06	52 (74.3)	0.53 (0.23–1.24)	0.15	P = 0.08

Variables	Sub-Variable	No of Ppt. n = 105	No of Abn smears n = 29	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 72	OR' (95% CI) Viral infection	P-value	MANOVA
Smokers	No	74	20 (27.0)	1		F = 0.73	50 (67.6)	1		F = 0.97
	Yes	31	9 (29.0)	0.84 (0.33–2.15)	0.72	P = 0.57	22 (71.0)	0.93 (0.38–2.22)	0.88	P = 0.45
Alcohol	No	56	17 (30.4)	1		F = 1.44	36 (64.3)	1		F = 6.26
	Yes	49	12 (24.5)	2.17 (0.90–5.26)	0.09	P = 0.23	36 (73.5)	1.79 (0.78–4.11)	0.17	P = 0.00*
HUTI	No	90	23 (25.6)	1		F = 1.81	60 (66.7)	1		F = 1.85
	Yes	15	6 (40.0)	0.49 (0.16–1.52)	0.21	P = 0.13	12 (80.0)	0.31 (0.13–1.91)	0.50	P = 0.10
Ulcer/Warts	No	94	24 (25.5)	1		F = 1.90	64 (68.1)	1		F = 1.36
	Yes	11	5 (45.5)	0.39 (0.11–1.39)	0.15	P = 0.12	8 (72.7)	0.80 (0.20–3.23)	0.75	P = 0.24
Vulva Itching	No	81	23 (28.4)	1		F = 4.89	54 (66.7)	1		F = 1.71
	Yes	24	6 (25.0)	1.12 (0.39–3.18)	0.83	P = 0.00	18 (75.0)	0.67 (0.24–1.87)	0.44	P = 0.13
Discharge	No	62	13 (21.0)	1		F = 2.59	43 (69.4)	1		F = 0.86
	Yes	43	16 (37.2)	0.50 (0.21–1.19)	0.12	P = 0.04*	30 (69.8)	0.91 (0.39–2.11)	0.83	P = 0.52
Vaginal bleeding	No	104	29 (27.9)	1		F = 0.09	72 (69.2)	1		F = 0.35
	Yes	1	0 (0.0)	0.00	1.00	P = 0.99	0 (0.0)	0.00	1.00	P = 0.91
Pain during sex	No	67	17 (25.4)	1		F = 1.41	45 (67.2)	1		F = 2.27
	Yes	38	12 (31.6)	0.68 (0.28–1.65)	0.39	P = 0.24	27 (71.1)	0.83 (0.35–1.98)	0.68	P = 0.04
Pelvic Pain	No	70	21 (30.0)	1		F = 1.36	49 (70.0)	1		F = 2.11
	Yes	35	8 (22.9)	1.35 (0.53–3.47)	0.53	P = 0.25	23 (65.7)	1.22 (0.51–2.89)	0.66	P = 0.06
Infection	No infection	33	3 (9.1)	1						
	EBV	3	0 (0.0)	0.04 (0.01–0.24)	0.00*					
	HPV	15	1 (6.6)	ORTL	1.00					
	HSV-2	21	6 (28.6)	0.03 (0.00–0.33)	0.00*					
	EBV + HSV-2	9	5 (55.6)	0.18 (0.04–0.81)	0.03*					
	HPV + HSV-2	11	5 (45.5)	0.56 (0.10–3.25)	0.51	F = 12.32				
	EBV + HPV + HSV-2	13	9 (69.2)	0.37 (0.07–1.97)	0.24	P = 0.00*				
Cervical integrity	Normal	76					44 (57.9)	1	0.66	
	Cervicitis	8					6 (75.0)	ORTL	1.00	
	ASCUS	8					7 (87.5)	ORTL	1.00	
	LSIL	9					9 (100)	ORTL	1.00	F = 11.35
	HSIL	4					4 (100)	ORTL	1.00	P = 0.00*

Variables	Sub-Variable	No of Ppt. n = 105	No of Abn smears n = 29	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 72	OR' (95% CI) Viral infection	P-value	MANOVA
Age at first sex	Less 18 years	62	16 (25.8)	1	0.60		39 (62.9)	1	0.33	
	19–21 years	39	11 (28.2)	0.35 (0.05–2.68)	0.31	F = 2.32	29 (74.4)	ORTL	1.00	F = 1.21
	22 and above	4	2 (50.0)	0.39 (0.05–3.15)	0.38	P = 0.06	4 (100)	ORTL	1.00	P = 0.31
Age at SWD	10–15 years	5	0 (0.0)	1	0.90		3 (60.0)	1	0.33	
	16–20 years	11	2 (18.2)	ORTL	1.00		5 (45.5)	0.50 (0.03–8.95)	0.64	

(*continued on next page*)

Table 2 (*continued*)

Variables	Sub-Variable	No of Ppt. n = 105	No of Abn smears n = 29	OR' (95% CI) Pap smear	P-value	MANOVA	No of Viral infection n = 72	OR' (95% CI) Viral infection	P-value	MANOVA
	21–25 years	46	16 (34.8)	0.22 (0.02–2.67)	0.24		34 (73.9)	0.19 (0.02–2.50)	0.21	
	26–30 years	27	9 (33.3)	0.53 (0.07–4.15)	0.55		20 (74.1)	0.85 (0.08–8.89)	0.89	
	31–35 years	12	0 (0.0)	2.50 (0.60–4.15)	0.52	F = 1.43	7 (58.3)	0.95 (0.09–10.73)	0.97	F = 0.30
	36–40 years	4	2 (50.0)	ORTL	1.00	P = 0.23	3 (75.0)	0.47 (0.04–5.90)	0.56	P = 0.94
Duration of sex work	0–2 years	56	21 (37.5)	1	0.40		40 (71.4)	1	0.44	
	3–4 years	25	3 (12.0)	ORTL	1.00		19 (76.0)	2.29 (0.30–17.66)	0.43	
	5–6 years	16	4 (25.0)	ORTL	1.00		9 (56.3)	3.17 (0.36–27.58)	0.30	
	7–8 years	3	0 (0.0)	0.00	1.00		1 (33.3)	0.00	1.00	
	9–10 years	1	1 (100.0)	ORTL	1.00	F = 0.82	1 (100)	2.00 (0.09–44.35)	0.66	F = 0.67
	11–12 years	4	0 (0.0)	0.00	1.00	P = 0.52	2 (50.0)	0.00	1.00	P = 0.67
No of Clients per month	1–30	83	23 (27.7)	1	0.68		60 (72.3)	1	0.27	
	31–60	12	2 (16.7)	0.51 (0.11–2.46)	0.40		8 (66.7)	0.29 (0.06–1.39)	0.12	
	61–90	3	1 (33.3)	0.27 (0.03–2.25)	0.22	F = 2.48	1 (33.3)	0.38 (0.06–2.56)	0.32	F = 1.25
	91–120	7	3 (42.9)	0.67 (0.04–11.29)	0.78	P = 0.05*	3 (42.9)	1.50 (0.09–25.39)	0.78	P = 0.29

Keys: OR; Odds ratio, ORTL; Odd ratio too large, CI; Confidence interval, MANOVA; Multivariate analysis, NC; Not calculated (calculation included empty cells), SWD; Sex work debut, N.F.E; No formal education, M.S.P; Multiple sexual partners, Sec; Secondary; D/S/W; Divorced/Separated/Widow, Abn; Abnormal, Ppt; participants, HUTI; History of urogenital infection; Uptake of cervical screening.

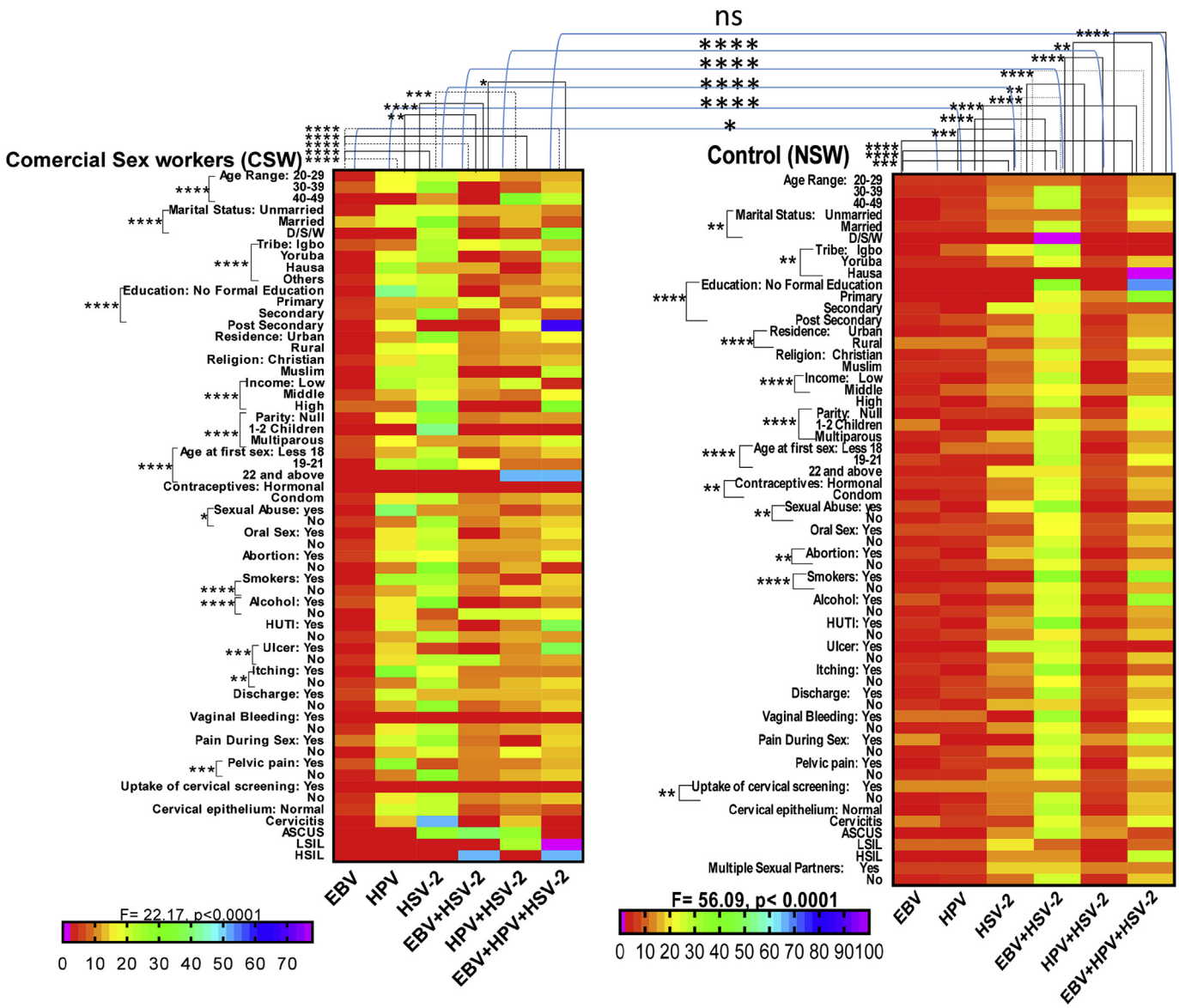

Fig. 2. Among CSWs and NSWs with respect to viral infections, the heatmap shows significant difference between the sub-variables in relation to marital status, tribe, education, income status, parity, age at first sex, sexual abuse and smoking ($p < 0.05$). Among CSWs, the figure shows significant difference between the sub-variables of age range, genital ulcer and itching around the genital (suggestive of ongoing infection) and pelvic pain ($p < 0.05$), while significant differences were observed between the sub-variables of contraceptives, abortion and uptake of cervical screening ($p < 0.05$; chi-square test) among NSW.

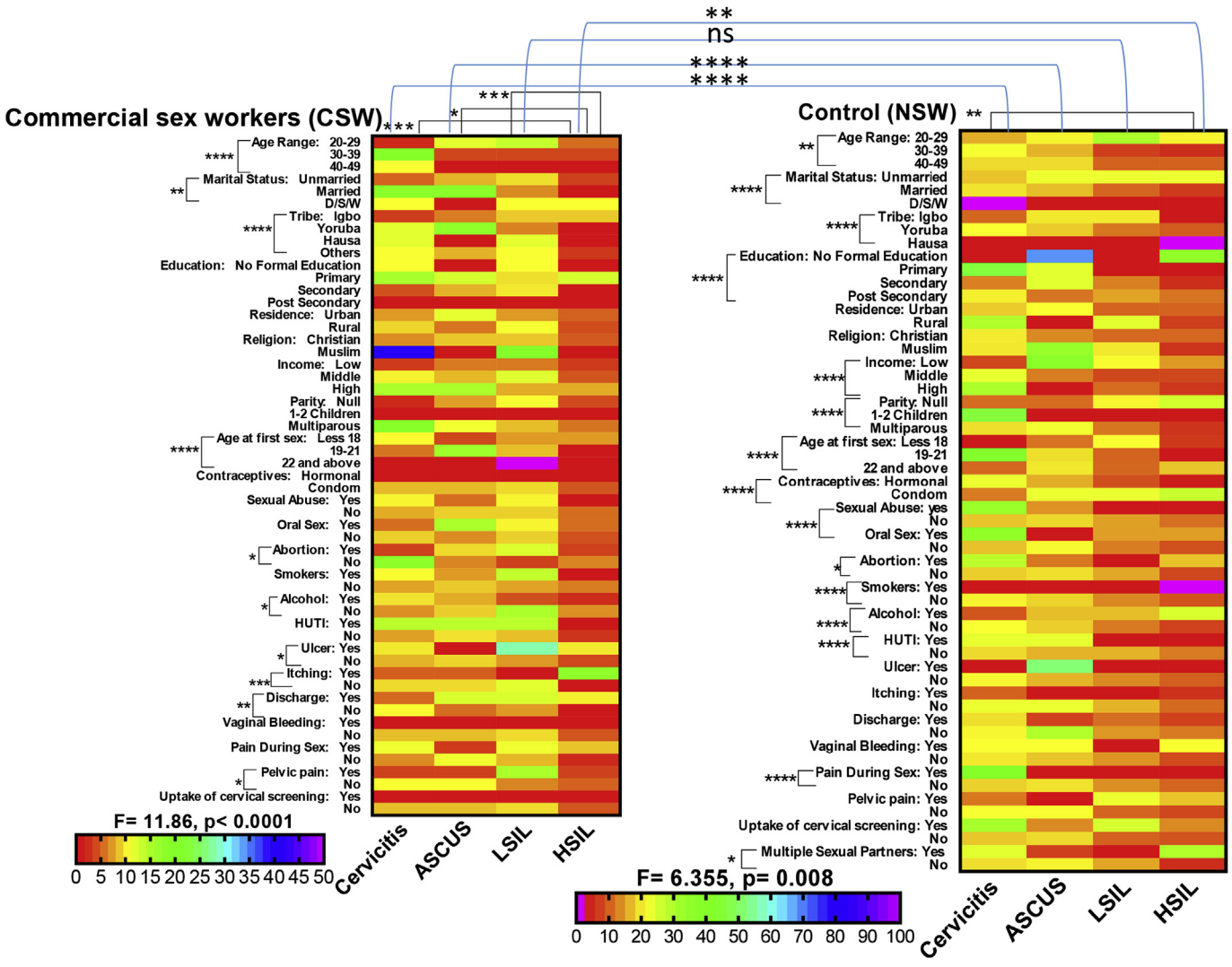

Fig. 3. Among sex workers and non-sex workers with respect to cervical abnormalities, the heatmap shows significant difference between the sub-variables in relation to age range marital status, tribe, abortion, alcohol and age at first sex ($p < 0.05$). Among sex workers, the figure shows significant difference between the sub-variables of itching around the genital and vaginal discharge (suggestive of ongoing infection), pelvic pain ($p < 0.05$), while significant differences were observed between the sub-variables of contraceptives, smokers, history of urogenital infection, pain during sex and multiple sexual partners ($p < 0.05$; chi-square).

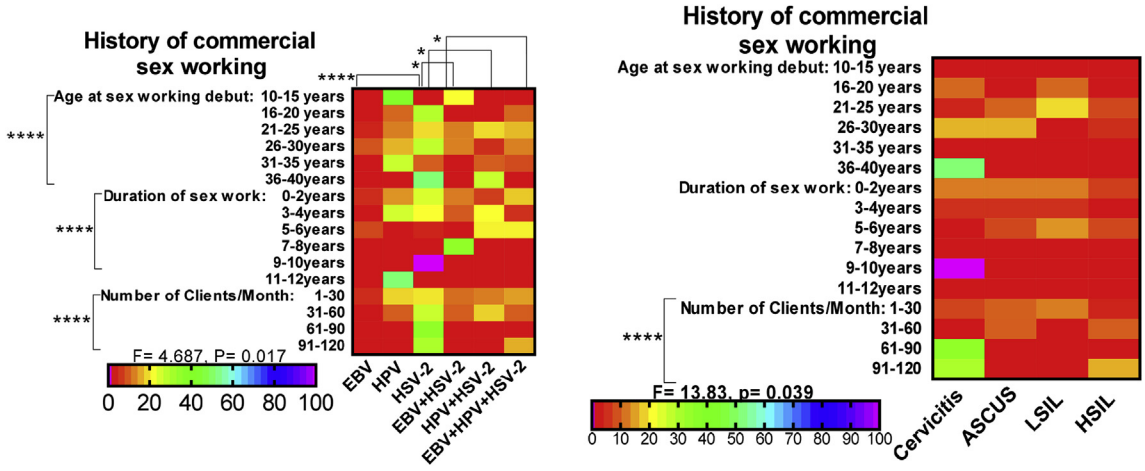

Fig. 4. Shows higher frequency of HSILs and cervicitis with higher number of sex clients per month. It also shows higher prevalence of HPV and HSV-2 mono-infection with increasing duration of sex work. It reveals higher prevalence of HPV mono-infection with early age of sexual intercourse (10–15 years).

compared with other types of infections in both groups at $p < 0.05$ (Fig. 2). Fig. 3 shows significant differences in the occurrence of cervical abnormalities (cervicitis, ASCUS and HSIL) between CSWs and NSWs ($p < 0.05$; *t*-test). It also reveals lower frequency of HSIL compared with other types of cervical abnormalities in both groups ($p < 0.05$). Result revealed higher HPV infection with early age of first sex and duration of sex work among CSWs. It also revealed higher frequency of viral co-infection and HSIL with higher number of clients (partners) per month among CSWs (Fig. 4). Fig. 5 showed higher and lower frequency of viral infections and cervical abnormalities among CSWs compared with NSWs ($p < 0.05$).

3.4. Expression of biomarkers and viral antibodies in relation to classes of Pap smears

Statistics showed similar pattern of increased serum expression of p16 in HPV related infections among NSWs and CSWs ($p > 0.05$) (Fig. 6). Non-sex worker with squamous intraepithelial lesions

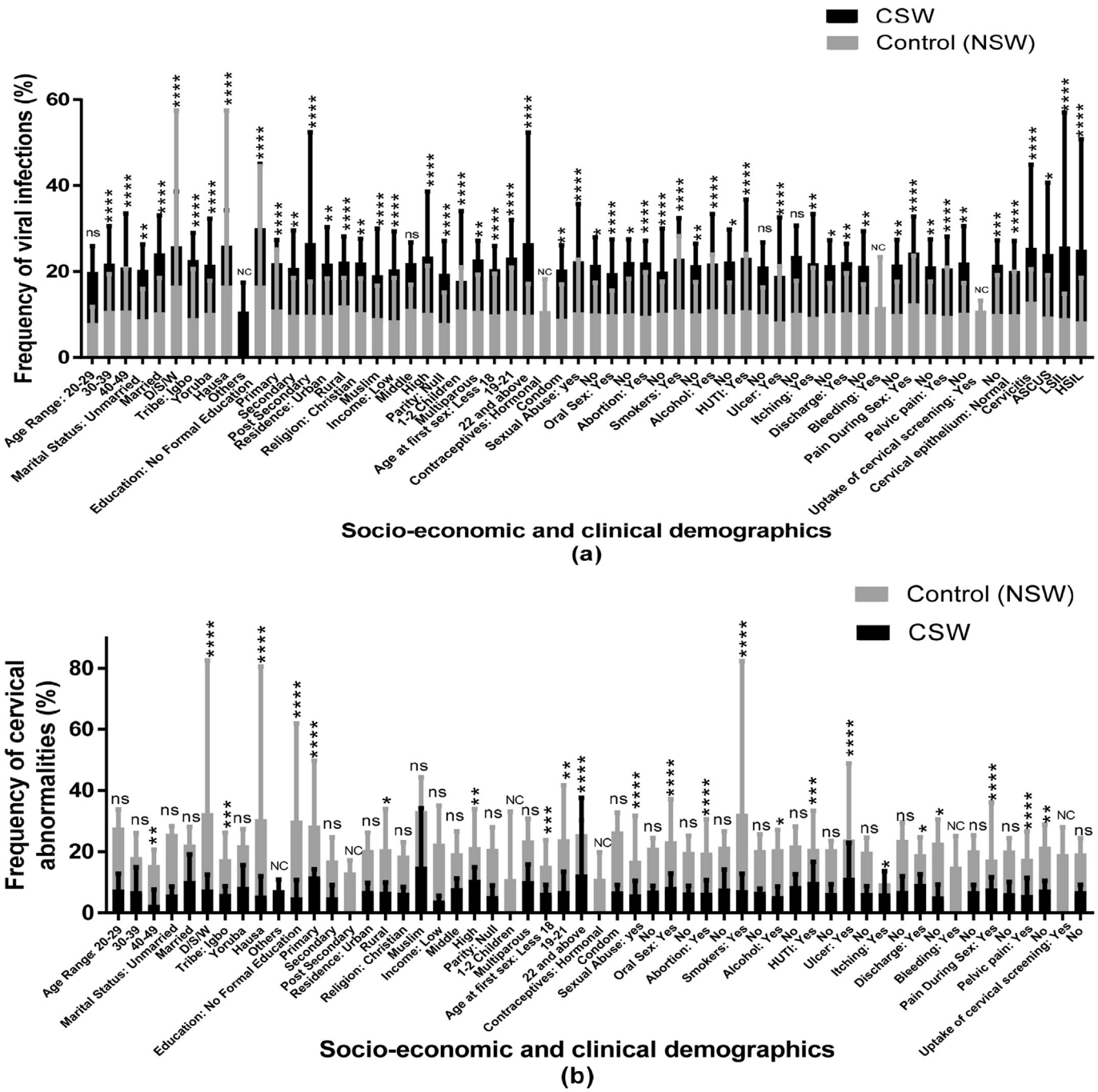

Fig. 5. Figure a shows higher frequency of viral infections among CSWs when compared with NSWs in relation to socio-clinical demographics, except in the age range of 20–29 years, middle income earners, those without HUTI and Ulcers ($p < 0.05$).
Figure 5b shows higher frequency of cervical abnormalities among NSWs when compared with NSWs in relation to age range of 40–49 years, D/S/W, Igbo and Hausa tribes, lack of formal education and primary school education, high income earners, first sex under the age of 19 years and above the age of 21 years, sex abuse, oral sex, abortion, smokers, HUTI, Ulcer, itching around the vulva, vaginal discharge, pain during sex and pelvic pain ($p < 0.05$).

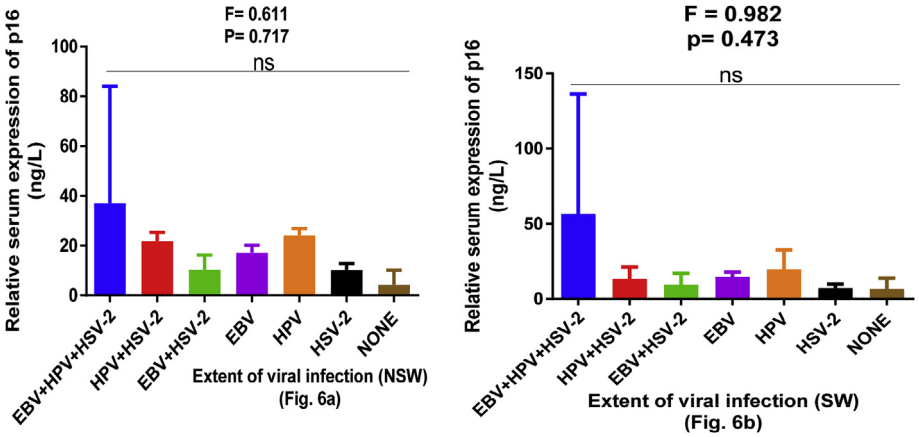

Fig. 6. Shows increased expression of p16 among NSWs and CSWs with EBV + HPV + HSV-2 tri-infection and HPV-mono-infections.

(SILs) had lower tissue ki67 and HPV positivity (55.6% and 50%) compared with CSWs (76.9% and 92.3%, respectively). Result showed significant correlation between HPV IgG antibody positivity and serum expression of p16 ($p = 0.04$) among CSWs. It also showed insignificant increase in serum expression of p16 among NSWs and SWs whose Pap smears were consistent with HSIL (Fig. 7). Statistics showed significant increase in serum expression of SCCA1 and ki67 with increasing severity of cervical abnormality among NSWs and CSWs, respectively (Figs. 8 and 9). Significant positive correlation was observed between serum levels of SCCA1 and Ki67 ($p = 0.01$) and EBV IgG and SCCA1 ($p = 0.02$) among NSWs. Among the study population with abnormal Pap smears (cervicitis, ASCUS, LSIL and HSIL), results showed higher serum SCCA1 positivity among NSWs (84.3%) compared with CSWs (82.4%), respectively while it shows lower serum Ki67 and p16 positivity among NSWs (62.7% and 47.4%, respectively) compared with CSWs (64.7% and 84.6%, respectively). When only ASCUS, LSIL and HSIL were considered, statistics showed p16 sensitivity and specificity of 64.7% and 79.5%, respectively (Odd ratio {OR} 0.7118), SCCA1 sensitivity and specificity of 83.8% and 97%, respectively (OR 166.9) and ki67 sensitivity and specificity of 63.2% and 94.0%, respectively (OR 26.83). It revealed that serum levels of SCCA1, ki67

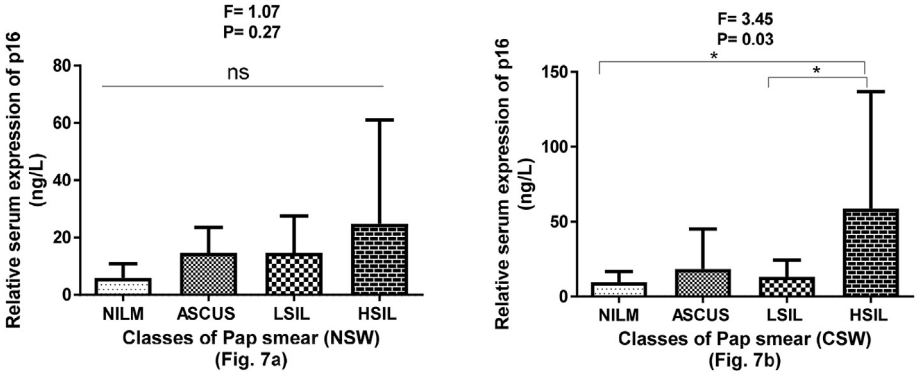

Fig. 7. Shows higher serum levels of p16 in Pap smears consistent with HSIL compared with other classes of Pap smear, especially among NSWs ($p < 0.05$).

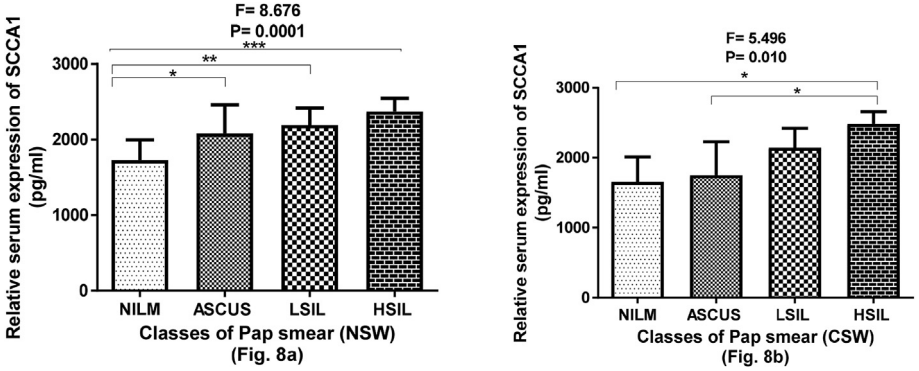

Fig. 8. Shows increasing serum levels of SCCA1 with increasing severity of cervical abnormality (from ASCUS to HSIL) when compared with NILM, especially among NSWs (8a) ($p < 0.05$).

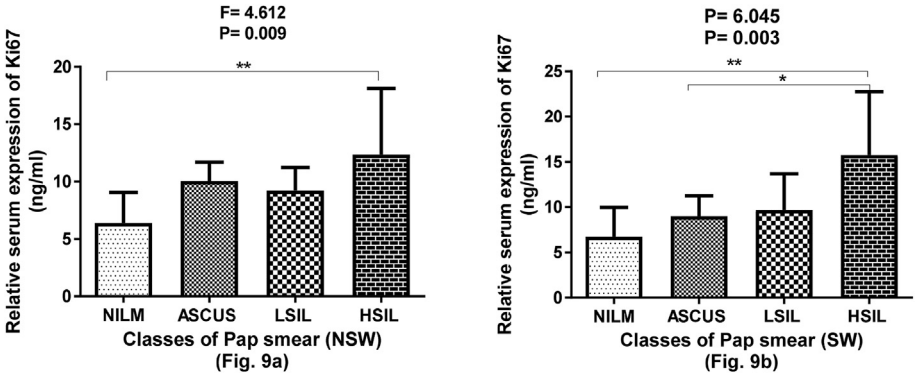

Fig. 9. Shows relative increasing serum levels of ki67 with increasing severity of cervical abnormality (from ASCUS to HSIL) when compared with NILM, especially among CSWs (9 b) ($p < 0.05$).

and p16 were sensitivity to the classes of Pap smear at $p = 0.00$; $p = 0.00$ and $p = 0.01$, respectively.

4. Discussion

This study assessed the prevalence of viral infection and abnormal Pap smears among CSWs and NSWs of reproductive age in relation to some sociodemographic characteristics. It also assessed expressions of some biomarkers in relation to the classes of Pap smears in a bid to reducing inter- and intra-observer variability.

Studies have shown that persistent infection with HPV is the strongest epidemiologic factor associated with cervical intra-epithelial neoplasia and cervical cancer.[15–17] This is in line with the findings of this study, especially among CSWs. In this study, the prevalence of viral infections decrease with age among CSWs while the prevalence of viral infections increase with age among NSWs. The higher prevalence of HPV infection and lower prevalence EBV infection observed among CSWs when compared with NSWs suggests that the observed difference may be a reflection of differences in sexual behaviour between the two groups. Among CSWs, the development of abnormal Pap smear and the acquisition of viral infections appeared to have been independently driven by HPV infection and alcohol consumption. Surprisingly, the higher prevalence of HPV infection among CSWs did not translate into higher prevalence of abnormal Pap smears when compared with NSWs. Since, higher frequency of HSILs and viral tri-infection were observed among CSWs who had greater than 90 clients, it is logical to say that increased sexual activity increases viral co-infection and cervical transformation. The higher abnormal Pap smears among NSWs, despite the lower prevalence of HPV infection, when compared with CSWs, suggests that EBV infection and some socio-economic factors may have played subtle or critical roles in the development of the abnormalities in the group. This is underscored by the correlation between chronic EBV infection and SCCA1 expression among NSWs. The consistent use of condom as a form of barrier contraceptive method may have lowered the acquisition of HPV infection and development of cervical abnormalities among CSWs. This agrees well with previous studies which posit that regular condom use lowers the rate of HPV infection among sexual partners, improve HPV clearance and promotes regression of cervical intraepithelial neoplasia.[18,19] The exclusion of sex workers living with HIV may also explain the lower prevalence of abnormal Pap smears recorded among CSWs in this study when compared with the prevalence recorded among similar population in Jos (Northern-Nigeria) and Nairobi, Kenya.[20,21] The low association of HSV-2 infection with abnormal Pap smear suggests that it may not have participated actively in the development of cervical epithelial transformation among CSWs and NSWs. The correlation between HSV-2 and EBV suggests that both viruses may favour the entry of each other during sexual activity, especially among NSWs who were most using non-barrier contraception. Though, HSV-2 and EBV-2 mono-infections and bi-infection had low p16 expression when compared with HPV mono-infection, their co-infection with HPV (tri-infection) had higher expression of p16. This suggest that HSV-2 and EBV mono-infections and bi-infection may directly or indirectly contribute to cervical carcinogenesis. The higher number of squamous cell intraepithelial lesions (SILs) associated with viral tri-infection also lend support to this fact. In other words, viral mono-infections and co-infections may have initiated and promoted the transformation of cervical epithelium, respectively. The lower expression of Ki67 among NSWs with SILs suggests higher chance of regression among the group.

The significant differences observed in serum levels of SCCA1 and ki67 in relation to severity of cervical abnormality suggest that they could differentiate one class of Pap smear from another better than p16 protein. The high sensitivity and specificity exhibited by SCCA1in relation to Pap smear classification also indicates that it is a better diagnostic marker compared with other evaluated biomarkers. Its correlation with ki67 suggests that both biomarker could be used as complementary biomarkers for screening and monitoring cervical abnormalities among CSWs and NSWs.

5. Conclusion

This study suggests that while alcohol consumption promotes viral infection among CSWs, HPV infection could independently promote the development of abnormal Pap smear in the group. It also suggests that EBV infection and other socio-economic demographics may play critical roles in the development of cervical abnormalities among NSWs. Lastly, this study suggests that serum ki67, p16 and SCCA1 expression could be used as Pap smear complementary diagnostic biomarkers.

Declarations of interest

None.

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